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Carbohydrate partitioning and sugar signalling in Cauliflower mosaic virus-infected turnip and Arabidopsis

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Abstract

We analyzed the correlation between the development of symptoms, virus titre and carbohydrate levels in turnip and Arabidopsis during infection with four isolates of *Cauliflower mosaic virus* (CaMV). Infection did not significantly affect sugar levels in source leaves except at the very late stages (28 d.p.i.), but induced a three-fold but short lived increase in sink leaves. Titres of severe isolates were greater than mild isolates, but we observed no obvious correlation between sugar levels and symptom appearance. In wild-type Arabidopsis, infection did not stimulate increased sugar levels, but did so in a mutant, *cnr160*, which shows altered growth-response to high carbon and low nitrogen. These results do not support a direct role for sugar-mediated control of symptom development during virus infection.

Keywords: Caulimovirus; Plant virus; Signalling mutants; Sugar repression; Sugar signalling

1. Introduction

Virus-infected plants typically develop symptoms which may include stunting, leaf distortions, dark greening and chlorosis, the latter often manifested as vein clearing and mosaics [1]. Large numbers of genes may be up- or down-regulated [2–5] and these changes in gene expression may take place in a co-ordinated manner depending on the progress of the infection [4,6]. Physiological changes include alterations in the levels and/or partitioning of metabolites between different tissues in the infected plant. Sugars have been reported to accumulate in photosynthetic source leaves of virus-infected plants. For example, in cotyledons of *Cucumber mosaic virus* (CMV)-infected marrow plants, soluble sugars were reported to accumulate, while levels of starch decreased [7,8]. Source leaves of CMV-infected melon plants showed elevated levels of free soluble sugars and reduced starch levels [9]. Changes in sugar partitioning might be attributable to several factors. CMV-infected marrow plants showed increased starch hydrolase and lowered ADP-glucose pyrophosphorylase activities, which might be expected to increase the conversion of starches into soluble sugars such as glucose, fructose and sucrose [7]. Reports implicate virus movement proteins in altering signalling networks controlling phloem loading and unloading [10], and transgenic plants expressing movement proteins of CMV and *Tobacco mosaic virus* (TMV) show increased accumulation of free soluble sugars in source leaves [9,11,12].

In yeast, sugar levels can influence mRNA transcription and translation rates [13], transcript stability [14,15] and protein turnover [16,17]. Such events are initiated after a signal(s) produced from hexose phosphorylation leads to the activation of transcription factors [18–20]. These modulate the expression of various genes in a manner that is dependent on the amount of sugar, and its rate of phosphorylation [21,22]. Carbohydrates also play a major role in gene regulation in plants [23] and it is thought that plants and yeast may utilize similar sugar signalling mechanisms [24–28]. Elevated sugars have been reported to increase levels of starch-related enzymes such as starch synthase [24], effecting conversion of soluble sugars into starch. Several sugar modulatable enzymes are involved in regulating sugar metabolism and partitioning [25]. Elevated sugar levels may regulate their own biosynthesis: for example,

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high levels of sugars are known to inhibit photosynthesis by repressing the expression of genes encoding proteins forming part of the photosynthetic apparatus such as chlorophyll a/b binding protein [26], Rubisco small subunit [27–29] and plastocyanin [26]. Accumulation of high levels of sugar may also lead to chlorophyll loss [30].

Sugars control gene expression through a complex series of pathways that also involve plant hormones including abscisic acid (ABA), ethylene, cytokinin and auxin [31,32]. Sugar-mediated regulation of gene expression has also been implicated in diverse processes that include wound-response, pigmentation and defence [33–40]. It is possible that perturbations in sugar partitioning that occur as a response to virus infection might affect sugar-regulated gene expression, altering, for example, chloroplast protein complexes or hormone ratios.

Recently, Martin et al. [41] demonstrated that carbon to nitrogen ratio rather than carbohydrate level alone appears to play a predominant role in regulating various aspects of seedling growth including photosynthetic gene expression. Based on growth medium conditions of 100 mM sucrose and 0.1 mM nitrogen [41] a number of *cnr* (carbohydrate non-responsive) mutants were isolated that did not show the wild-type response of unexpanded, purple cotyledons after 6–8 days growth [42]. The absence of a response in wild-type Arabidopsis, when grown on 0.1 mM nitrogen supplemented with different osmotica suggests that the response to sucrose is not due to osmotic stress.

Virus-induced elevations in sugar levels and the consequent effects on pathways involved in pigmentation and hormone function, provide a plausible mechanism to explain the development of symptoms, in particular those involving altered colouration and leaf distortion. Evidence to support this hypothesis comes from experiments by Von Schaewen et al. [30] in which sugars were forced to accumulate in transgenic tobacco over-expressing a yeast invertase. Plants showed a mosaic and stunted phenotype, reminiscent of virus symptoms. Herbers et al. [43] over-expressed the movement protein from *Potato leafroll virus* (PLRV) in tobacco: this gave rise to major changes in sugar partitioning and starch accumulation and also induced a symptom-like phenotype. Possibly, increases in sugar levels in localized regions of the leaf may lead to chlorosis. Neighbouring cells may produce more green pigments to compensate for the loss in carbon fixation, resulting in mosaics.

To investigate the possible link between sugars and the development of symptoms, we infected turnips with four isolates of *Cauliflower mosaic virus* (CaMV), which induce symptoms ranging from very severe (strong mosaic patterning, chlorosis, severe stunting) to very mild (whole plant dark green colouration, with no stunting or distortions). Emergent and expanded tissues were harvested at intervals after inoculation, and viral DNA, starch and total soluble sugars were assayed. Since sugar signalling in turnip is not well elucidated, we additionally used Arabidopsis since it is a compatible host for CaMV and a number of *cnr* (carbohydrate non-responsive) mutants are available [48]. To further analyze any possible link between carbohydrate status and the development of symptoms, we infected wild-type Arabidopsis and three *cnr* mutants with a severe isolate of CaMV. Tissue was harvested just as symptoms were developing, and levels of sugar, starch and virus accumulation were measured. Although we were able to identify virus-dependent changes in sugar and starch levels in turnip and in wild-type and mutant Arabidopsis, we found no obvious correlation between changes in sugar levels and the development of symptoms.

2. Materials and methods

2.1. Virus infection

CaMV isolates used in this work were Aust [44,45], Baji-31 [46,47], Bari-1 [48,49] and Cabb B-JI [45,49]. Virus was maintained and propagated in turnip (*Brassica rapa-rapifera* cv. 'Just Right') as described by Cecchini et al. [46]. Infection studies were carried out in a controlled environment room. Turnip plants (cv. 'Just Right') were grown in compost at a temperature of 22°C. Light was provided by Osram 'Warm White' fluorescent tubes at an intensity of 100 μmol m^{-2} s^{-1} for 16 h per day. Plants were manually inoculated with 1 μg purified virus as described [46]. Infection studies on Arabidopsis (*Arabidopsis thaliana*) were carried out in a similar environment except that the illumination period was 10 h per day. Plants were grown in compost and inoculated manually with 100 ng purified virus after emergence of the first true leaves, as described [46]. Mutants *cnr160*, *cnr171*, and *cnr181*, have been described [41].

2.2. Assay of virus

Virus levels in infected turnips were estimated by quantitating virus DNA using slot-blot hybridization [51]. Leaf tissue was harvested in triplicate (each replicate comprising tissue from 4 plants) from fully expanded leaves (approximately 10–20 cm in length—representing source tissue for photosynthates [54]) and unexpanded leaves (approximately 1–2 cm long—representing sink tissue for photosynthates [54]) and stored at −80°C. DNA was extracted from approximately 100 mg of tissue using a Puregene kit (Flowgen, Shenstone, Staffs, England) according to the manufacturer's protocol, except that after grinding in extraction buffer, proteinase K was added at a final concentration of 250 μg mL^{-1} and samples were incubated for 3 h at room temperature to release genomic DNA from virus capsids [50]. DNA concentration was estimated spectrophotometrically. Using a vacuum blot apparatus, aliquots containing 100, 10 and 1 ng of denatured DNA from each sample were loaded onto Hybond N nylon membranes (Amersham, Bucks, England) as described [3].

Hybridization was carried out as described previously using as probe ^{32}P-labelled insert DNA from plasmid pUC-BJI which carries the complete coding sequence for CaMV gene VI [51]. Blots were exposed to X-ray film, lightly exposed autoradiographs were scanned and digitized using a flatbed scanner,

and levels of hybridization calculated by pixel analysis using QuantiScan for Windows (Biosoft, Cambridge, England) as described [52].

Level of viral replication in infected Arabidopsis was estimated by quantitating virus 35S + 19S RNA by Northern blot hybridization [52]. Leaf tissue was harvested in triplicate (each replicate being derived from 4 plants) from fully expanded leaves or emergent leaves (length 1–2 mm) and stored at −80°C. These sizes were selected as representing sources or sinks for photosynthates according to the criteria of Imlau et al. [53]. Total RNA was extracted using a PureScript Kit (Flowgen, Shenstone, Staffs, England), and approximately 2 μg was separated by electrophoresis on agarose-formaldehyde mini-gels. RNA was transferred to Hybond N membrane (Amersham, Bucks, England) and virus-specific RNA was detected by Northern blot hybridization using a ^{32}P-labelled insert DNA from pUC-BJI as probe. Lightly exposed autoradiographs were scanned, digitized, and hybridization was quantitated by pixel analysis using QuantiScan for Windows. To correct for any lane-to-lane variations in the quantities of RNA loaded, for each lane, the value for hybridizing RNA was corrected by the value for total RNA loaded (quantitated from ethidium bromide fluorescence) as described [52].

2.3. Starch and soluble sugar extraction and analysis

Free soluble sugar and starch levels were measured in infected plants and in uninoculated controls maintained in parallel. Samples were taken in triplicate from 12 plants, each sample being derived from tissue pooled from 4 plants. Arabidopsis tissue samples were taken from whole expanded and emergent (unexpanded) leaves. For turnip, 1 cm-diameter discs were removed from emergent and expanded leaves, with care being taken to avoid leaf veins. Tissues were ground under liquid nitrogen, 250 μL 80% (v/v) ethanol was added and then incubated for 1 h at 70°C. Samples were subjected to three successive 10 min centrifugations in a microcentrifuge. The pellets were retained for starch analysis. The pooled supernatants were lyophilized, re-dissolved in 50 μL of 100 mM imidazole pH 6.9, 5 mM $MgCl_2$. Free soluble sugar concentrations in the samples were determined using an enzyme linked assay [54].

Five microlitres of extract or standards (5 mM glucose, fructose or sucrose) were added to 191 μL of 100 mM imidazole pH 6.9, 5 mM $MgCl_2$, and 2 μL 100 mM ATP in microtitre plate wells. Two microlitres of 200 mM NADP and 0.4 U of glucose-6-phosphate dehydrogenase were added and the absorbance at 340 nm was read in a BioRad Benchmark microplate reader (Bio-Rad Laboratories, Hemel Hempstead, UK). One microlitre each of hexokinase (0.3 U), phosphoglucose isomerase (0.3 U) and invertase (0.8 U) were then added to each well, the plate incubated at 37°C and A_{340} was measured after 10 min. The total concentration of glucose, fructose and sucrose was measured and expressed in terms of glucose units using an equation adapted from Stitt et al. [54].

2.4. Statistical analysis of data

Statistical analyses were carried out using Minitab for Windows (v. 9.0). Data were analyzed by ANOVA using the General Linear Model (GLM) and Bonferroni analysis. In order to avoid a type1 error in the Arabidopsis data, for mutants derived from Col-0 or WS-0, GLM was carried out separately on an ecotype basis.

3. Results

3.1. Symptom phenotypes of different CaMV isolates in turnip

Turnips were infected with four CaMV isolates which induce symptoms ranging from very severe to very mild; Aust > Cabb B-JI > Baji 31 > Bari [44–46]. Chlorotic local lesions appeared on the inoculated leaves of all virus-inoculated plants at 7 days post-inoculation (d.p.i.). At 14 d.p.i. mosaic patterning, mild stunting and leaf malformation had developed in turnips inoculated with Aust and Cabb B-JI. Baji-31-infected plants developed mild mosaics, but no stunting or leaf malformation; Bari-1-infected plants showed no systemic symptoms.

At 21 d.p.i., symptoms were very apparent (Fig. 1). Stunting and mosaic patternation were very extensive in turnips inoculated with Aust or Cabb B-JI, but very mild with Baji-31. Plants infected with Bari-1 showed no obvious stunting or mosaics but displayed an unusual dark greening throughout the entire plant. By 28 d.p.i. the emergent leaves of Aust-infected plants were very severely stunted and most of the plants had died by 35 d.p.i. Although Cabb B-JI produced severe symptoms in turnips, the stunting and malformation were not quite as extensive as those observed in Aust-infected

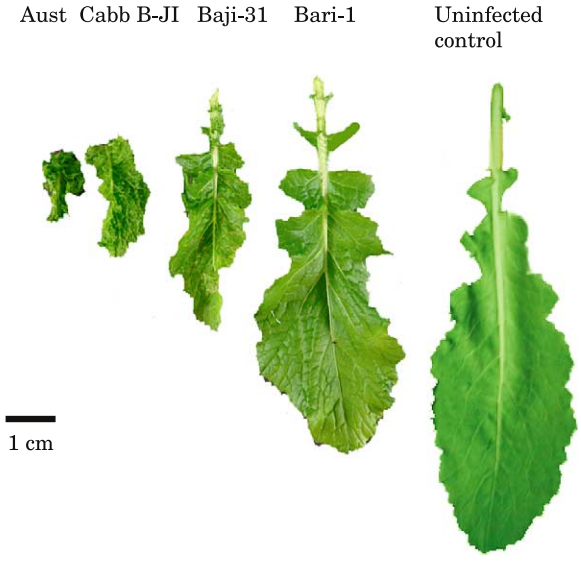

Fig. 1. Stunting and mosaic patternation in leaves of turnip plants infected with CaMV isolates Aust, Cabb B-JI, Baji-31 and Bari-1, compared with uninfected control. At the time of inoculation, plants were of similar size and stage of development. All photographs are at the same magnification. The leaves shown are all at the same stage of development (leaf no. 7) and were excised from infected plants at 21 d.p.i. One centimetre scale bar is shown.

plants. Baji-31 produced symptoms comparable to those seen with Cabb B-JI 7 days earlier, distinct mosaic patternation and some stunting. Bari-1-infected plants were similar in size to uninfected plants and developed dark green leaves without any mosaics. These observations are similar to those reported previously [45,46].

3.2. Virus accumulation in expanded and unexpanded turnip leaves

To test whether differences in virus levels might be responsible for variations in symptom severity, virus DNA was measured in leaf samples at 7 day intervals after inoculation (Fig. 2). All four virus isolates had spread systemically by 14 d.p.i. and virus DNA was present in both fully expanded (10–20 cm in length) and unexpanded (1–2 cm in length, barely emergent and folded) leaves. Since the sink-source transition does not take place until after leaves have unfolded [55], these represent photosynthetic source and sink tissue at the time of sampling. Virus levels were greatest in plants infected with the two more severe isolates (Aust and Cabb B-JI), whereas with milder isolates Bari-1 and Baji-31, accumulation was significantly lower ($p < 0.05$). Levels of the two more severe isolates in fully expanded leaves were highest at 21 d.p.i. In contrast, the milder isolates Bari-1 and Baji-31 reached their maximum levels at 14 d.p.i. In unexpanded leaves, all virus isolates except Bari-1 were at their greatest levels at 14 d.p.i., and declined gradually thereafter.

Although the more severe isolates accumulated to greater levels, neither virus levels nor distribution showed a strict correlation with either the severity or timing of symptoms. For example, levels of Aust and Cabb B-JI in corresponding leaves were not significantly different, although symptoms in Aust-infected plants were consistently more severe, particularly at 21 and 28 d.p.i. Also stunting and mosaic patternation in sink leaves of plants infected with the three most severe isolates increased progressively from 14 to 28 d.p.i., although virus levels were maximal at 14 to 21 d.p.i.

3.3. Soluble sugar and starch accumulation in infected and uninfected turnip

To identify any correlation between soluble sugars, symptom severity and virus accumulation, sugar levels were measured in infected and uninfected plants at 7, 14, 21 and 28 d.p.i. (Fig. 3). In uninfected plants, both fully expanded (sources of photosynthates) and unexpanded leaves (sinks) showed a steady increase in the level of total soluble sugars with time. With one exception, sugar levels in expanded leaves of infected and uninfected plants were not significantly

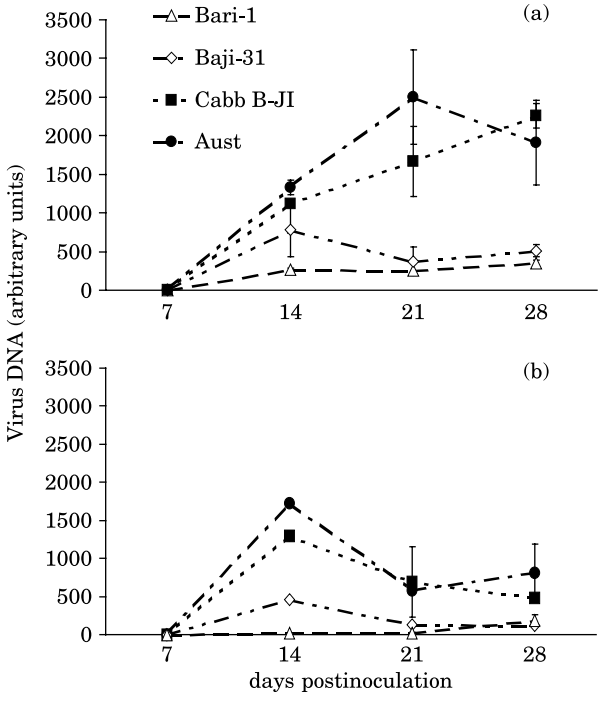

Fig. 2. Levels of CaMV DNA in (a) fully-expanded leaves, (b) unexpanded leaves of turnip at 7 day intervals after inoculation with four isolates of CaMV. Total DNA was extracted from leaves as described in Section 2. Levels of virus DNA were determined by slot-blot hybridization using ^{32}P-labelled DNA from pUC-BJI as a probe. Blots were exposed to X-ray film for 24 h and hybridization was quantititated by pixel analysis of autoradiographs as described. Virus DNA is expressed in arbitrary units. Error bars indicate standard deviations ($n = 3$). Triplicate samples were taken from 12 plants, each sample comprising pooled tissue of 4 plants.

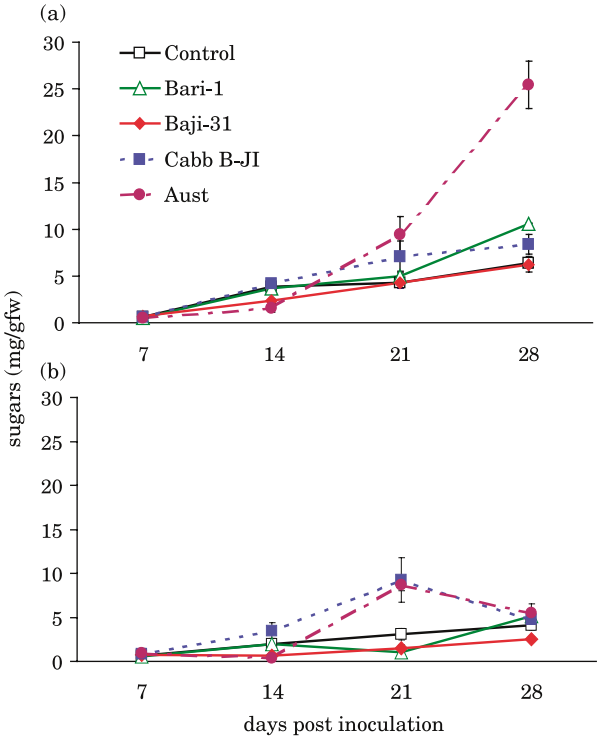

Fig. 3. Levels of free soluble sugars in (a) fully-expanded leaves, (b) unexpanded leaves of turnip at 7 day intervals after inoculation with four isolates of CaMV, and in uninfected control plants grown in parallel. Sugar levels were determined as described in Section 2 and are expressed as mg of (glucose + fructose + sucrose) per g fresh weight (gfw) of leaf tissue. Error bars indicate standard deviations ($n = 3$). Triplicate samples were taken from 12 plants, each sample comprising pooled tissue of 4 plants.

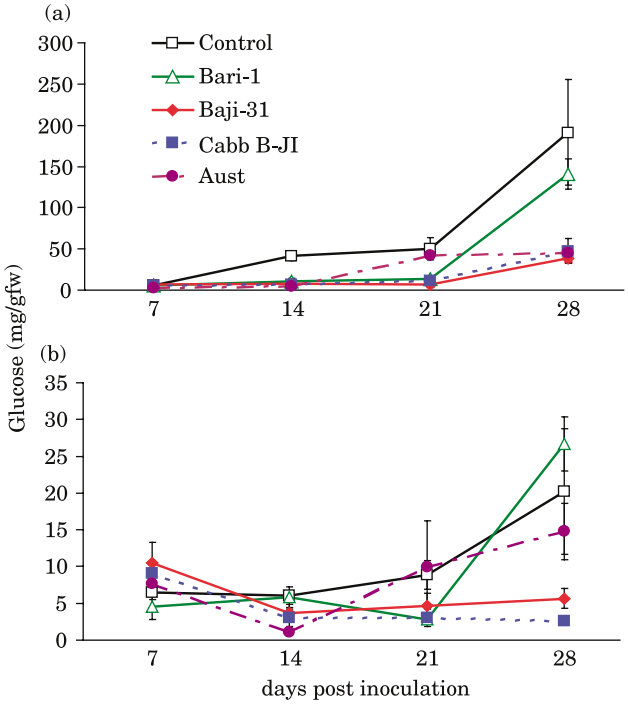

Fig. 4. Levels of starch in (a) fully-expanded leaves, (b) unexpanded leaves of turnip at various times after inoculation with four isolates of CaMV, and in uninfected control plants grown in parallel. Starch levels were determined as described in Section 2 and are expressed as glucose equivalents (mg per g fresh weight of leaf tissue). Error bars indicate standard deviations ($n=3$). Triplicate samples were taken from 12 plants, each sample comprising pooled tissue of 4 plants.

different ($p>0.05$; Fig. 3a). However at 28 d.p.i., sugar levels in Aust-infected expanded leaves were consistently elevated by more than four-fold (significant at $p<0.05$) compared to plants infected with the other isolates and uninfected controls. In unexpanded (sink) leaves we also detected no significant differences ($p>0.05$) between sugar levels in uninfected plants and plants infected with isolates Bari-1 and Baji-31. However, compared to uninfected controls, levels of total soluble sugars in unexpanded leaves of plants infected with both Aust and Cabb B-JI showed a three-fold elevation at 21 d.p.i. (Fig. 3b). The rise and then fall of sugar levels in unexpanded leaves of Aust and Cabb B-JI infected plants at 21 d.p.i. was statistically significant ($p<0.05$). These changes were reproducible in three independent experiments.

In uninfected plants, starch levels increased with age (Fig. 4). For all four virus isolates, starch levels in source leaves were generally significantly ($p<0.05$) lower than in uninfected controls of the same age. At 28 d.p.i. starch levels in source leaves of plants infected with the three most severe isolates were approximately one quarter that of uninfected controls, although with Bari-1, starch levels were not significantly different ($p>0.05$) from the uninfected controls (Fig. 4a). Sink leaves of uninfected plants contained much less starch than source leaves. Infection had no significant effect on starch levels up to 21 d.p.i. ($p>0.05$). However by 28 d.p.i., starch levels in turnip infected with Cabb B-JI and Baji-31 isolates were significantly ($p<0.05$) lower than uninfected controls (Fig. 3b).

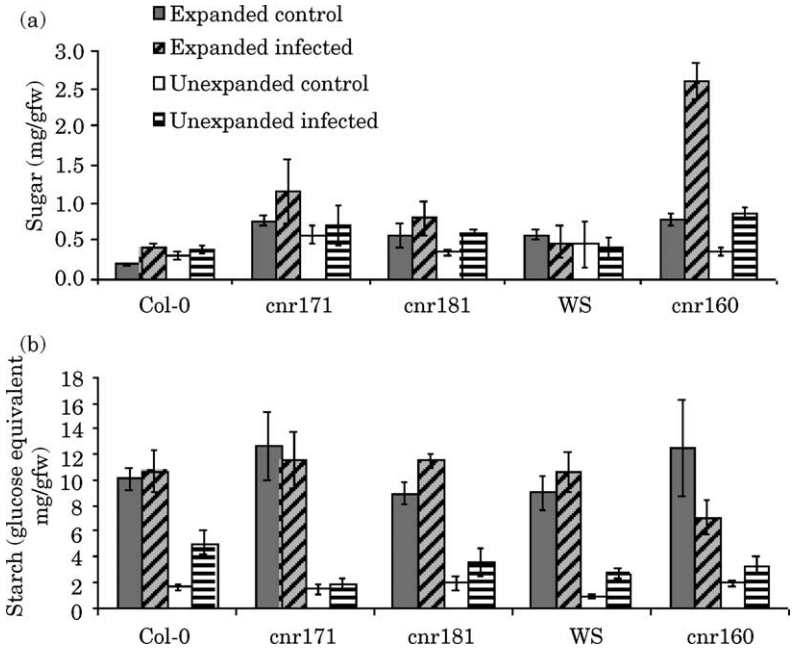

Fig. 5. Levels of (a) free soluble sugars and (b) starch in expanded and unexpanded (emergent) leaves of uninfected and infected wild-type Arabidopsis and *cnr* mutants, and in CaMV Cabb B-JI-infected plants at 14 d.p.i. Sugar levels were determined as described in Section 2 and are expressed as mg of (glucose + fructose + sucrose) per g fresh weight (gfw) of leaf tissue. Starch levels are expressed as glucose equivalents (mg per gram fresh weight of leaf tissue). Error bars indicate standard deviations ($n=3$). Triplicate samples were taken from 12 plants, each sample comprising pooled tissue of 4 plants.

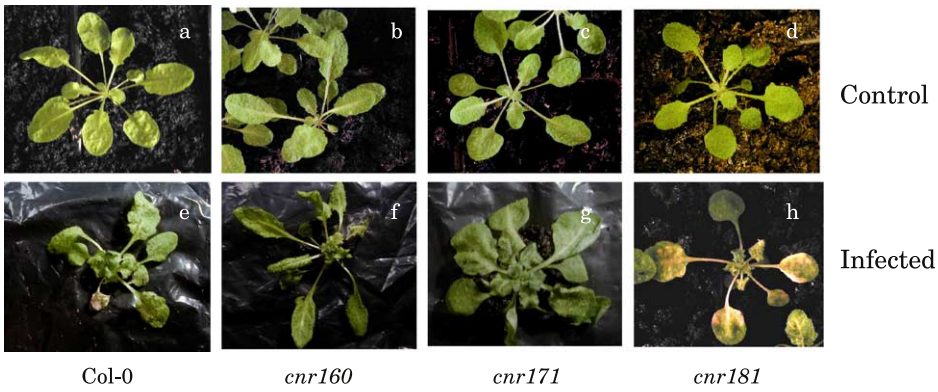

Fig. 6. Symptom expression in wild-type and mutant Arabidopsis, 21 days after inoculation with CaMV Cabb B-JI. Mock-inoculated controls are shown for comparison. (a) Col-0, uninfected; (b) *cnr160* uninfected; (c) *cnr171* uninfected; (d) *cnr181* uninfected: (e) Col-0, infected; (f) *cnr160* infected; (g) *cnr171* infected; (h) *cnr181* infected. Symptoms in infected WS-0 were identical to those in Col-0 (not shown).

3.4. CaMV pathogenesis and carbohydrate levels in wild-type and Arabidopsis sugar response mutants

Three of these mutants, *cnr160*, *cnr171*, and *cnr181* and wild-type Arabidopsis, grown in parallel and at the same stage of growth, were inoculated with CaMV. Isolate Cabb B-JI was used because it gives the most consistent symptom development in Arabidopsis [46]. *cnr160* is in a WS-0 background, whereas *cnr171* and *cnr181* are in a Col-0 background [42], wild-type controls were from the appropriate background. Sugar, starch and virus levels were measured at 14 d.p.i. We selected this time-point because systemic symptoms become visible to the naked eye at 12 to 13 d.p.i. [46], and we would have expected any changes in sugar levels that were responsible for inducing chlorosis or other symptoms to have been detectable at this stage in the infection process. In fact, infection did not significantly alter sugar levels in wild-type Arabidopsis ($p > 0.05$) although it did induce a significant ($p < 0.05$) two to three-fold increase in starch levels in unexpanded leaves, representing sink tissue [53] (Fig. 5a and b).

Two of the mutants showed an altered symptom response to CaMV infection, *cnr171* developing much less severe stunting and mosaics compared to Col-0 and *cnr181* developing very severe stunting and a pink colouration (Fig. 6). Symptoms in *cnr160* were similar to those in wild-type (Fig. 6). Sugar levels in all leaves of *cnr171* and *cnr181*, infected and uninfected, were elevated two to three-fold compared to the corresponding leaves from Col-0 (Fig. 5; significant at $p < 0.05$). However, multifactorial ANOVA indicates that these differences are attributable to the effect of the genetic background: infection itself is not a significant factor. We detected no significant differences ($p > 0.05$) between levels of total soluble sugars in uninfected *cnr160* and WS-0. However, infection induced a highly significant ($p < 0.01$) three-fold rise in sugar levels in expanded- and a two-fold increase ($p < 0.05$) in unexpanded leaves in the mutant, not seen in wild-type (Fig. 5).

In *cnr160,* infection also resulted in a two-fold decrease in starch levels in expanded leaves ($p < 0.05$). In contrast, infection had little effect on starch levels in expanded (source) leaves of any of the other Arabidopsis genotypes. However, the infection-dependent increase in starch accumulation observed in unexpanded leaves from wild-type plants was significantly reduced ($p < 0.05$) in *cnr171* and *cnr181*.

To test whether the alterations in the symptom responses and sugar levels were a consequence of altered virus load, levels of virus 35S plus 19S RNA (a measure of actively replicating virus) were measured at 14 d.p.i. (Fig. 7). ANOVA indicates no significant differences ($p > 0.05$) between levels of viral RNA in wild-type (Col-0 and WS) and two of the mutants, *cnr181* and *cnr171*, although symptoms in *cnr171* were milder and in *cnr181* were more severe than in wild-type plants. In contrast, levels of viral RNA in both expanded and emergent leaves of *cnr160* (which developed similar symptoms to wild-type plants, but accumulated high levels of free soluble sugars in response to infection) were four-fold higher than in WS-0 (highly significant at $p < 0.01$).

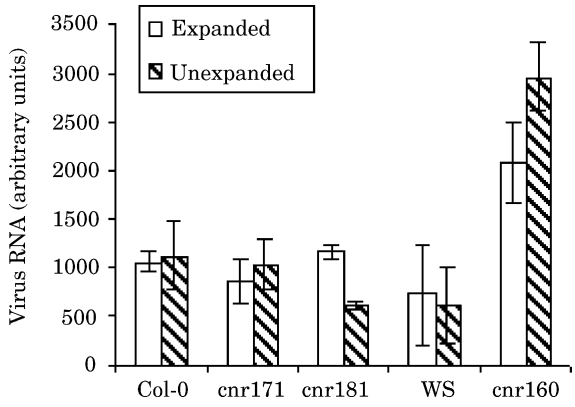

Fig. 7. Levels of CaMV (isolate Cabb B-JI) RNA in wild-type Arabidopsis and *cnr* mutants at 14 d.p.i. Total RNA was extracted from leaves as described in Section 2. Levels of virus 35S + 19S RNA were determined from Northern blot hybridization using ^{32}P-labelled DNA from pUC-BJI as a probe. Blots were exposed to X-ray film for 24 h and hybridization was quantititated by pixel analysis of autoradiographs as described. Virus RNA is expressed in arbitrary units and is corrected for lane differences in loading by normalizing to rRNA. Plain bars indicate expanded leaves, hatched bars indicate unexpanded (emergent) leaves. Error bars indicate standard deviations ($n = 3$). Triplicate samples were taken from 12 plants, each sample comprising pooled tissue of 4 plants.

4. Discussion

Although in turnip we observed a broad correspondence between virus load and symptom severity, isolate-specific differences in symptom severity, for example between Aust and Cabb B-JI and between Baji-31 and Bari-1, cannot be attributed to differences in virus levels alone. Also, two of the Arabidopsis mutants *cnr171* and *cnr181*, showed altered symptom responses although virus levels were similar to wild-type, whereas a third mutant, *cnr160*, in which symptoms were of similar severity to wild-type, accumulated levels of actively replicating virus that were four times that in wild-type. These observations confirm and extend our earlier findings [52] that there is no simple direct link between symptom severity and virus titre. Rather, symptoms develop through a complex series of host responses, and depend on genetic variations within the host or pathogen and the developmental state of the host [46,51,52,56,57].

We analyzed changes in levels of free soluble sugars and starch in CaMV-infected turnip, and in Arabidopsis mutants altered in response to high sucrose and low nitrogen. It has been suggested that infection might induce changes in the levels of sugars, and that perception of such changes by the plant would result in the down- (or up-) regulation of genes encoding for example, components of the photosynthetic apparatus, enzymes involved in various metabolic pathways and defence-related proteins [58]. This is a plausible explanation for the appearance of symptoms such as chlorosis, whole plant dark greening, stunting and leaf malformation.

Our observations do not support such a simple mechanism, at least in the case of infection by CaMV. One prediction of the model is that increases in sugar levels should coincide with or precede the development of symptoms. Also, virus isolate-specific differences in the severity of chlorosis induced in infected plants should be reflected in differences *in planta* in levels of soluble sugars. Neither of these predictions is met. In general, levels of free soluble sugars in source leaves of infected turnip did not differ significantly from levels in uninfected controls. Sink leaves of turnip infected with the two most severe isolates, Aust and Cabb B-JI, but not the milder isolates Baji-31 and Bari-1, showed a transient but statistically significant increase in sugar levels at around 21 d.p.i. However, these changes lagged behind the appearance of chlorotic mosaics and there was no obvious correlation between symptom severity and sugar levels (c.f. Cabb B-JI and Bari-1 in source leaves). In wild-type Arabidopsis and two of the *cnr* mutants, we did not observe any significant effect of infection on sugar levels. At 14 d.p.i. symptoms are developing rapidly and we would certainly have predicted significant differences between sugar levels in infected and uninfected plants at this stage of infection had sugar signalling been contributing significantly to symptom development.

It is more likely that changes in sugar levels form part of the host response to infection. In *Red clover mottle virus strain O* (RCMV-O)-infected pea plants, atypical large starch grains accumulate in the chloroplast [59]. Although RCMV-O infects plants systemically, symptoms include necrosis at the actively growing apex. This necrosis effectively removes the sink, allowing sugar to accumulate and may be responsible for the subsequent increase in starch levels [59]. The extreme stunting of the apical leaves in Aust-infected turnip may bear a similar responsibility for the increase in sugar levels late in infection.

Several reports have linked virus-induced increases in sugar levels with increased accumulation of starch [60,61]. In other reports, soluble sugars accumulated, while levels of starch decreased; CMV infection of marrow plants, increased starch hydrolase and lowered ADP-glucose pyrophosphorylase activities [7,10]. This might be expected to increase the conversion of starches into soluble sugars, such as glucose, fructose and sucrose. We found that CaMV-infection in turnip, in particular, the isolates that accumulated to the highest levels, Aust and Cabb B-JI, suppressed the age-dependent accumulation of starch that occurred in both source and sink leaves of uninfected plants. Tecsi et al. [7] have suggested that the inhibition of starch accumulation and/or starch degradation might be a consequence of the increased demand for soluble sugars, required to maintain the respiration rate and support viral replication; this may be the case with CaMV-infected turnips. In contrast, in wild-type Arabidopsis, sugar levels were unaffected by infection, although there was a significant increase in starch accumulation in sink leaves at 14 d.p.i. Differences between turnip and Arabidopsis might reflect the much lower CaMV titres [46], and consequent lower metabolic demand for virus replication, in the latter.

All three Arabidopsis mutants differed from each other in their responses to infection by CaMV. Although all were identified in a screen designed to select potential sugar signalling mutants, they show pleiotropic phenotypes, and the nature of the lesions are unknown. Nevertheless it may be significant that all three showed altered responses to infection. Although carbohydrate levels are not involved in symptom development in wild-type Arabidopsis or turnip, Arabidopsis sugar signalling mutants showed altered responses to infection. Our observations are inconsistent with a simple mechanistic hypothesis, but do not exclude the possibility of more complex or subtle roles for sugar signalling in the responses to infection.

In plants, the partitioning of sugars between sinks and sources is constantly changing, and the resulting perturbations (which may manifest themselves as unusual sugar deficits or surpluses), are believed to be short lived [25]. Because we measured levels of carbohydrates and virus accumulation in excised leaves (Arabidopsis) or leaf discs (turnip), transient changes, or changes localized to particular tissue types or regions within the leaf would not have been easily identified in our experiments. Indeed spatial and temporal differences in metabolism and gene expression are a feature of spreading virus infections [6,7,62]. Nevertheless, where we did identify significant changes in sugar levels, for example in the Arabidopsis *cnr160* mutant and in turnips infected with Aust and Cabb B-JI, we were unable to identify any obvious correlation with the development of symptoms such as chlorosis. Thus, gross changes in sugar partitioning such as that reported in transgenic plants expressing invertase or virus

movement protein [11,30,43] are unlikely to be the immediate drivers of symptom expression during CaMV-infection.

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